



STATE OF MARYLAND

DHMH

Maryland Department of Health and Mental Hygiene

Larry Hogan, Governor - Boyd Rutherford, Lt. Governor - Dennis R. Schrader, Secretary

Laboratories Administration
Robert A. Myers, Ph.D., Director

DATE: January 9, 2017

TO: Medical Laboratory Directors, Local Health Officers, and Health Care Providers

FROM: Robert A. Myers, Ph.D.
Director, Laboratories Administration

RE: **Updated** Guidance and Instructions for Submission of Specimens for Suspected Zika Virus Infection Testing at the Maryland DHMH Laboratory (January 2017)

On Monday, January 9, 2017, the Maryland DHMH Laboratory will begin using the CDC developed Trioplex multiplex real-time PCR (RT-PCR) assay to detect Zika virus RNA in clinical specimens that have been pre-authorized for testing. The Trioplex assay is approved for use by the federal Food and Drug Administration (FDA) under an Emergency Use Authorization (EUA) and has the added advantage of being able to simultaneously screen certain acute specimen types for both chikungunya and dengue virus RNA. The DHMH Laboratory has performed extensive verification and validation studies in preparation for this test transition and found that the Trioplex assay demonstrated similar performance characteristics when compared to the existing single-plex Zika, dengue and chikungunya RT-PCR assays that are currently in use in our laboratory.

The procedures for preapproval of Zika testing at the DHMH Laboratory remain unchanged (*see attached Guidelines and Instructions for Zika Testing*) and testing of at-risk patients will continue to be performed in accordance with the most current CDC and DHMH guidance and algorithm (<http://phpa.dhmh.maryland.gov/pages/zika.aspx>). However, the types and volumes of specimens used in the Trioplex assay will be somewhat different (*see attached Guidelines and Instructions for Zika Testing; Specimens Collection and Handling Table*). The Trioplex assay has been validated and approved for use with larger volumes of serum which improves the sensitivity of the assay. Additionally, whole blood (purple top tube: EDTA anticoagulant) and cerebrospinal fluid (CSF) can also be tested in the Trioplex assay. Some studies have indicated that testing of whole blood during the acute phase of Zika infections might improve the detection of viral RNA. **The use of plasma for Zika RT-PCR at the DHMH Laboratory is now discontinued.** Please be aware that a serum specimen must be submitted along with the other specimen types that can be tested in the Trioplex PCR assay. **Urine, CSF and whole blood specimens received without an accompanying serum cannot be tested in the Trioplex assay.**

The DHMH Laboratory will continue to test serum specimens using the CDC developed FDA EUA approved Zika IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA) for the presence of IgM antibodies. Extensive cross-reactivity in flavivirus serological assays has been documented. Therefore, if specimens are reactive in the Zika MAC-ELISA an additional paired convalescent serum might be required to complete additional plaque reduction neutralization testing (PRNT) testing at the CDC Laboratory to possibly identify the most recently infecting flavivirus.

Contact the DHMH Molecular Diagnostic Laboratory at (443) 681-3924 or (443) 681-3905 during normal business hours (8:00AM to 4:30 PM weekdays) for questions regarding the transition to the Trioplex assay for Zika virus RNA testing and the Arbovirus Serology Laboratory at (443) 681-3937 or (443) 681-3932 for inquiries related to Zika virus IgM testing.

Howard Haft, MD, MMM, CPE, FACPE
David Blythe, MD, MPH

PLEASE NOTE – REVISED CHART (01-10-2017)

Maryland Department of Health and Mental Hygiene Laboratories Administration
Guidelines and Instructions for Zika Testing

A) Preapproval of Zika Test Requests

An infectious disease consultation with a Local Health Department (LHD) or DHMH is still required to authorize specimens for Zika virus testing at the MD DHMH Laboratory, prior to submitting specimens. Contact your Local Health Department or the DHMH Infectious Disease Epidemiology and Outbreak Response Bureau at (410) 767-6700 (or after hours, at (410) 795-7365) for consultation. Prior to contacting the LHD or DHMH, review of the current interim CDC guidance found in the link below, is highly recommended. <http://phpa.dhmmh.maryland.gov/pages/zika.aspx>

B) Specimen Collection and Handling

Testing Category	Specimen Type (see notes 1 & 2)	Volume/Amount	Collect in:	Storage and Shipping Conditions (see note 3 for storage >5 days)
Symptomatic Adults and Children Asymptomatic Pregnant Women <i>Refer to http://www.cdc.gov/zika/hc-providers/types-of-tests.html</i>	Serum	3-5ml (6-10 ml blood draw)	Red top, tiger top, or gold top serum separator tube	Refrigeration (2-8°C)
	Whole Blood	4-5 ml	Purple top EDTA tube	Refrigeration (2-8°C)
	Urine	5-10 ml	Leak proof, sterile urine cup; label as urine	Refrigeration (2-8°C)
	Cerebral Spinal Fluid (CSF)	1-2 ml	Leak proof, sterile tube or vial; label as CSF	Refrigeration (2-8°C)
Infants (within 2 days of birth) <i>Refer to CDC Guidelines on Collecting and Submitting Specimens at Time of Birth for Zika Virus Testing http://www.cdc.gov/zika/hc-providers/test-specimens-at-time-of-birth.html</i>	Serum	≥2 ml serum (≥4 ml blood draw)	Red top, tiger top, or gold top serum separator tube	Refrigeration (2-8°C)
	Urine	5-10 ml	Leak proof, sterile urine cup; label as urine	Refrigeration (2-8°C)
	Fresh Placenta, Fetal Membranes, Umbilical Cord	1 inch square of: - Umbilical cord - Fetal membranes - Placental disk edge - Placental disk midsection - Pathologic Lesions	Clearly labeled sterile cup with lid tightly closed; place each specimen in individually labeled cup	Refrigeration (2-8°C)
	Fixed Placenta, Fetal membrane, Umbilical Cord	1 inch square of: - Umbilical cord - Fetal membranes - Placental disk edge - Placental disk midsection - Pathologic Lesions	Fix specimens in formalin; volume of formalin used should be as small as possible, but about 10x mass of tissue.	Room Temperature

Notes:

- 1) A serum specimen must accompany all urine, CSF or whole blood specimens, or testing will not be performed.
- 2) Plasma will no longer be accepted for Zika testing at DHMH.
- 3) If specimens (except whole blood and fixed tissue) are to be held for longer than 5 days after collection until delivery to the testing lab, it is recommended to freeze to ≤20°C and ship frozen (on dry ice). Avoid repeated freezing and thawing cycles. Whole blood EDTA should not be frozen but refrigerated and tested within one week of collection. Fixed tissues should be held and shipped at room temperature.

For detailed instructions on how to submit other specimen types (including amniotic fluid and semen) for Zika, dengue, chikungunya and other arboviral tests, contact the MD DHMH Laboratories at (443) 681-3924 or (443) 681-3937 during normal business hours from 8:00 a.m. - 4:30 p.m., Monday through Friday.

For detailed instructions about collecting and submitting specimens, refer to the attached instructions on the DHMH Serological Testing Request Form No. 4677 Sample Form or go to the MD DHMH Laboratories website (*insert replacement website for updated guidance doc*). This form **must** be completed when submitting pre-approved specimens for all Zika virus test requests to the DHMH Laboratory. Specimens submitted without this form or without prior approval from the Health Department will NOT be accepted for testing. **Please ensure that all required core demographic, provider, and patient contact information is completed.** In addition, please provide the following information to facilitate testing. Failure to include the additional clinical and epidemiological information will delay testing.

- a. **Name of Health Department Person Approving Testing:** Please record on the requisition the name of the DHMH or local health department person approving the testing.
- b. **Clinical Illness/Compatible clinical presentation:** e.g., rash, acute onset fever, conjunctivitis, arthralgia
- c. **Pertinent travel history:** Recent travel to a region where local transmission of Zika virus has been documented (an updated list is available at <http://www.cdc.gov/zika/geo/index.html>)
- d. **History of any previous flavivirus infection:** e.g., West Nile virus (WNV), dengue virus
- e. **Acute illness onset date:** contemporaneous with the travel exposures in areas of ongoing transmission (illness onset date ≤ 14 days after exposure)
- f. **Immunization history:** Yellow fever (YF), Japanese encephalitis (JE), or Tick-borne encephalitis (TE) vaccines

D) Shipping:

Specimens collected for Zika virus testing can be transferred within the U.S. as Category B Biological substances in accordance with Department of Transportation (DoT) Hazardous Materials Regulations (49 CFR Part 171-180). Guidance for packaging samples in accordance with Category B Biological substance requirements can be found in the CDC/NIH Publication Biosafety in Microbiological and Biomedical Laboratories, 5th edition. Additional information about the DoT Hazardous Materials Transport Regulations can be found at <https://www.transportation.gov/pipelines-hazmat>. Appropriately packaged specimens can be directly shipped via a public carrier to the MD DHMH Laboratories Administration at the following address:

Maryland DHMH Laboratories Administration
1770 Ashland Avenue
Baltimore, MD 21205

Contact your local health department for alternative arrangements to have specimens forwarded to the MD DHMH Laboratory. The DHMH Laboratory has routine couriers who pick up specimens from most of the local health departments on weekdays for delivery to the central lab in Baltimore for testing.

During normal business hours (8:30AM to 4:30 PM weekdays), for questions regarding the transition to the Triplex assay for Zika virus RNA testing, please contact the DHMH Molecular Diagnostic Laboratory at 443-681-3924 or 443- 681-3905 or the Arbovirus Serology Laboratory at 443-681-3937 or 443-681-3924 for inquiries related to Zika virus IgM testing.

Must complete the test request authorization information (This is where reports will be sent). Include the name of Healthcare provider who can legally order the test(s) in “Test Request Authorized by”

Request Arbovirus Travel-Associated Panel. Provide specimen source:

Indicate “S” for serum –
(SST or aliquot) or whole
clotted blood (red top)

Accompanying
specimens*:

Indicate "B" for whole unclotted blood with EDTA (Purple top)
UNSPUN

Indicate “U” for urine. (Leak-proof sterile urine cup)

Indicate “CSF” for Cerebrospinal fluid (Leak-proof sterile tube or vial)

***Urine, Whole blood, and CSF
MUST be submitted with an
accompanying serum
specimen.**

Complete patient's Travel history (**location and dates**), symptoms (or asymptomatic), **pregnancy status (including weeks of gestation)**, vaccination history, & immune status

For questions on Zika Virus testing, please contact the lab:
PCR: (443) 681-3923/3924
Serology: (443) 681-3932/3937

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Laboratories Administration MD DHMH
1770 Ashland Ave. • Baltimore, MD 21205
443-681-3800 <http://dhhm.maryland.gov/laboratory>
Robert A. Myers, Ph.D., Director

SEROLOGICAL TESTING

<input type="checkbox"/> EH <input type="checkbox"/> FP <input type="checkbox"/> MT/YPN <input type="checkbox"/> NOD <input type="checkbox"/> STD <input type="checkbox"/> TB <input type="checkbox"/> CD <input type="checkbox"/> COR		Patient SS# (last 4 digits) _____	
Health Care Provider Address _____ City _____ County _____ State _____ Zip Code _____ Contact Name: _____ Phone# _____ Fax# _____		Last Name _____ First Name _____ Date of Birth (mm/dd/yyyy) _____ / ____ / ____ Address _____ City _____ State _____ Zip Code _____	
Test Request Authorized by: _____ Sex: <input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Transgender M to F <input type="checkbox"/> Transgender F to M Race: <input type="checkbox"/> American Indian/Alaska Native <input type="checkbox"/> Asian <input type="checkbox"/> Black/African American <input type="checkbox"/> Native Hawaiian/Other Pacific Islander <input type="checkbox"/> White MRN/Case # _____ DOC # _____ Outbreak # _____ Date Collected: _____ Time Collected: _____ Previous Test Done? <input type="checkbox"/> no <input type="checkbox"/> yes Name of Test: _____ Name of Test: _____ Onset Date: _____ Exposure Date: _____		*Specimen Source Code* Herpes Simplex Virus (HSV) Types 1&2 Legionella Leptospira Lyme Disease *WBC/Immunity Screen*: (Measles (Rubella), Mumps, Rubella, Varicella (Chickenpox) IgG Ab only) Mononucleosis - infectious *Mumps Immunity Screen Mycoplasma Rocky Mountain Spotted Fever (RMSF) *Rabies (RFFIT) (List vaccination dates above) *Rubella Immunity Screen *Rubella (Measles) Immunity Screen Schistosoma Strongyloides Syphilis - Previously treated? <input type="checkbox"/> yes <input type="checkbox"/> no Toxoplasma Tuberculosis Venereal Immunity Screen VDRL (CSF only) CDC/Other Tests: _____ Add Specimen Codes: _____	
Specimen Source Code Arbovirus Panels (Serum or CSF) Mandatory: Onset Date, Collection Date, and Travel History Arbovirus Endemic Panel (WNV, EEE, SLE, LAC) Arbovirus Travel-Associated Panel (Chikungunya, Dengue) Based on information provided PCR and/or immunological assays will be performed. Required information, check all that apply: DIAGNOSIS: <input type="checkbox"/> Jeptic Meningitis; <input type="checkbox"/> Encephalitis <input type="checkbox"/> Other: _____ SYMPTOMS: Headache, Fever, Light neck stiffness, mental state, muscle weakness, rash Other: _____ ILLNESS FATAL? <input type="checkbox"/> yes <input type="checkbox"/> no TRAVEL HISTORY (dates and places): _____ IMMUNIZATIONS: Yellow fever? <input type="checkbox"/> yes <input type="checkbox"/> no Polio? <input type="checkbox"/> yes <input type="checkbox"/> no MUMUNOCOMPROMISED? <input type="checkbox"/> yes <input type="checkbox"/> no		*Specimen Source Code* *LAVENDER TOP TUBE REQUIRED* Hemoglobin Disorders Blood transfusion? (last 4 months) <input type="checkbox"/> yes <input type="checkbox"/> no Prenatal screen? <input type="checkbox"/> yes <input type="checkbox"/> no Failure of baby screen? <input type="checkbox"/> yes <input type="checkbox"/> no Guardian's name if patient is a minor: _____ Name of mother of "at risk" baby: _____ RESTRICTED TEST Pre-Approved Submitters Only Submit a separate specimen for HIV Instructions go to: http://doh.maryland.gov/laboratory HIV Country of Origin: _____ Rapid Test: <input type="checkbox"/> Reactive <input type="checkbox"/> Negative Date: _____ Specimen stored refrigerated (2-8°C) after collection. <input type="checkbox"/> yes <input type="checkbox"/> no Specimen transported on cold packs <input type="checkbox"/> yes <input type="checkbox"/> no	
Specimen Source Code Epstein-Barr Virus (EBV) Hepatitis A Screen (IgM Ab only, acute infection) Call lab (443-581-3889) prior to submitting Hepatitis B Screen (HBs antigen only) Prenatal patient? <input type="checkbox"/> yes <input type="checkbox"/> no *Hepatitis B Panel (HBsAg, HBsAb) *Hepatitis B post vaccine (HBsAb) *Hepatitis C screen (HCV Ab only)		*Specimen Source Code* B Blood (5 ml) CSF Cerebrospinal Fluid L Lavender Top Tube P Plasma S Serum (1 ml per test) UR Urine	
Zika Virus Approved by: ####		Prior arrangements have been made with the following DHMH Labs Administration Approved by: ####	

Patient's first & last names must be on the specimen container and exactly match the lab slip

Collection Date and Onset of symptoms Date **MUST** be completed

If specimens other than whole blood, urine, serum, or CSF are being requested, please note type of specimen here, e.g.:

Fresh or Fixed Tissue

You must write “Zika Virus” to request testing

Include the name of the Local Health Department or DHMH Epidemiologist who approved testing

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443-681-3800 <http://dhmh.maryland.gov/laboratories/>
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SEROLOGICAL TESTING

<input type="checkbox"/> EH <input type="checkbox"/> FP <input type="checkbox"/> MTY/PN <input type="checkbox"/> NOD <input type="checkbox"/> STD <input type="checkbox"/> TB <input type="checkbox"/> CD <input type="checkbox"/> COR			
Health Care Provider		Patient SS# (last 4 digits):	
Address		Last Name <input type="checkbox"/> SR <input type="checkbox"/> JR <input type="checkbox"/> Other	
City	County	First Name	M.I.
State	Zip Code	Date of Birth (mm/dd/yyyy)	/ /
Contact Name:		Address	
Phone#	Fax#	City	County
Test Request Authorized by:		State	Zip Code
Sex: <input type="checkbox"/> Male <input type="checkbox"/> Female <input type="checkbox"/> Transgender M to F <input type="checkbox"/> Transgender F to M		Ethnicity: Hispanic or Latino Origin? <input type="checkbox"/> yes <input type="checkbox"/> no	
Race: <input type="checkbox"/> American Indian/Alaska Native <input type="checkbox"/> Asian <input type="checkbox"/> Black/African American <input type="checkbox"/> Native Hawaiian/other Pacific Islander <input type="checkbox"/> White			
MRN/Case #	DOC #	Outbreak #	Submitter Lab #
Date Collected:	Time Collected:	<input type="checkbox"/> am <input type="checkbox"/> pm	*Vaccination History: _____
Previous Test Done? <input type="checkbox"/> no <input type="checkbox"/> yes		Name of Test	Date <input type="checkbox"/> 1st <input type="checkbox"/> 2nd <input type="checkbox"/> 3rd State Lab Number: _____
		Name of Test	Date <input type="checkbox"/> 1st <input type="checkbox"/> 2nd <input type="checkbox"/> 3rd State Lab Number: _____
Onset Date: _____		Exposure Date: _____ <input type="checkbox"/> Clinical Illness/Symptoms: _____	

↓ SPECIMEN SOURCE CODE Arbovirus Panels (Serum or CSF) Mandatory: Onset Date, Collection Date, and Travel History <div style="border: 1px solid black; padding: 2px; margin: 2px;">Arbovirus Endemic Panel (WNV, EEE, SLE, LAC)</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Arbovirus Travel-Associated Panel (Chikungunya, Dengue)</div> <p>Based on information provided PCR and/or immunological assays will be performed.</p> <p>Required information, check all that apply: DIAGNOSIS: <input type="checkbox"/> Aseptic Meningitis <input type="checkbox"/> Encephalitis <input type="checkbox"/> Other _____</p> <p>SYMPTOMS: <input type="checkbox"/> headache <input type="checkbox"/> fever <input type="checkbox"/> stiff neck <input type="checkbox"/> altered mental state <input type="checkbox"/> muscle weakness <input type="checkbox"/> rash <input type="checkbox"/> Other _____</p> <p>ILLNESS FATAL? <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>TRAVEL HISTORY (dates and places) _____</p> <p>IMMUNIZATIONS: Yellow fever? <input type="checkbox"/> yes <input type="checkbox"/> no Flavivirus? <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>IMMUNOCOMPROMISED? <input type="checkbox"/> yes <input type="checkbox"/> no</p> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Aspergillus</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Chlamydia (group antigen IgG)</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Cryptococcal (antigen)</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Cytomegalovirus (CMV)</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Ehrlichia</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Epstein-Barr Virus (EBV)</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Hepatitis A Screen (IgM Ab only, acute infection) Call lab (443-681-3889) prior to submitting</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Hepatitis B Screen (HBs antigen only) Prenatal patient? <input type="checkbox"/> yes <input type="checkbox"/> no</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">*Hepatitis B Panel: (HBsAg, HBsAb)</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">*Hepatitis B post vaccine (HBsAb)</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Hepatitis C screen (HCV Ab only)</div>	↓ SPECIMEN SOURCE CODE <div style="border: 1px solid black; padding: 2px; margin: 2px;">Herpes Simplex Virus (HSV) Types 1&2</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Legionella</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Leptospira</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Lyme Disease</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">*MMRV Immunity Screen: (Measles (Rubeola), Mumps, Rubella, Varicella (Chickenpox) IgG Ab only]</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Mononucleosis - Infectious</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">*Mumps Immunity Screen</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Mycoplasma</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Rocky Mountain Spotted Fever (RMSF)</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">*Rabies (RFFIT) (*List vaccination dates above)</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">*Rubella Immunity Screen</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">*Rubeola (Measles) Immunity Screen</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Schistosoma</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Strongyloides</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Syphilis - Previously treated? <input type="checkbox"/> yes <input type="checkbox"/> no</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Toxoplasma</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Tularemia</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Varicella Immunity Screen</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">VDRL (CSF only)</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">CDC/Other Test(s)</div> <p>Add'l Specimen Codes _____</p> <p>Prior arrangements have been made with the following DHMH Labs Administration employee: _____</p> <p>_____</p> <p>_____</p> <p>_____</p> <p>Please Note Vaccination History above*</p>	↓ SPECIMEN SOURCE CODE <div style="border: 1px solid black; padding: 2px; margin: 2px; color: blue;">* LAVENDER TOP TUBE REQUIRED</div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">Hemoglobin Disorders</div> <p>Blood transfusion? (last 4 months) <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>Prenatal screen? <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>Father of baby screen? <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>Guardian's name if patient is a minor: _____</p> <p>Name of mother of "at risk" baby: _____</p> <div style="border: 1px solid black; padding: 5px; margin: 10px; text-align: center;"> RESTRICTED TEST Pre-Approved Submitters Only Submit a separate specimen for HIV Instructions go to: http://dhmh.maryland.gov/laboratories/ </div> <div style="border: 1px solid black; padding: 2px; margin: 2px;">HIV</div> <p>Country of Origin _____</p> <p>Rapid Test: <input type="checkbox"/> Reactive <input type="checkbox"/> Negative</p> <p>Date: _____</p> <p>Specimen stored refrigerated (2°-8°c) after collection. <input type="checkbox"/> yes <input type="checkbox"/> no</p> <p>Specimen transported on cold packs <input type="checkbox"/> yes <input type="checkbox"/> no</p> <div style="border: 1px solid black; padding: 2px; margin: 2px;"> SPECIMEN SOURCE CODE: PLACE CODE IN BOX NEXT TO TEST B Blood (5 ml) CSF Cerebrospinal Fluid L Lavender Top Tube P Plasma S Serum (1 ml per test) UR Urine </div>
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